Exercise within LBNP as an Artificial Gravity Countermeasure

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A. INTRODUCTION

1. SUMMARY PARAGRAPH

Recent results suggest musculoskeletal structure and function depend importantly on gravitational blood pressures and flows. This dependency may be one reason why exercise countermeasures for prolonged microgravity have failed to prevent loss of bone and muscle during prolonged space flight. Previously we found that lower body negative pressure (LBNP) produces a "footward" reaction force at the feet equal to the product of the pressure differential inside to outside the LBNP chamber and the body cross-sectional area at the waist seal. Static force approximately equivalent to one Earth body weight is generated against the feet by each 50 mmHg of LBNP either during standing or supine posture. Further, subjects exercising against this level of LBNP have biomechanical and cardiovascular responses similar to 1g. Current concepts of bungee cord-assisted, treadmill exercise are limited by harness discomfort, lower than normal loads, abnormal postflight gait, and the absence of gravitational blood pressures within the vascular system of the lower extremities. Our long-term objective is to provide an efficacious and integrated physiologic countermeasure for prolonged space flight in terms of cost, crew time, mass, volume, and power usage.

2. PROJECT AIMS, OBJECTIVES, AND HYPOTHESES

The long-term objective of this research is to test the concept of load-bearing exercise within a LBNP chamber to maintain musculoskeletal and other physiologic systems during long-term exposure to microgravity. We believe development of this integrated countermeasure will provide: 1) a more physiologic level and type of biomechanical stress than previously-proposed countermeasures for long-duration space flight, 2) sufficient and comfortable loading to maintain structure and function of musculoskeletal and other tissues, and 3) a safe, cost effective, reduced crew time, low power, small mass and small volume alternative to artificial gravity by centrifugation for many physiologic systems.

Specific Objectives for Achieving the Long-Term Goal:

1) In women volunteers, determine whether a daily regimen of 40 min of treadmill running at 1-1.2 body weights followed by 5 min of static LBNP prevents bed-rest deconditioning as well as it has done so in men.
2) Redesign LBNP exercise chamber as flight hardware.

3. CRITICAL ROADMAP QUESTIONS ADDRESSED

Our project is relevant to the Biomedical Research and Countermeasures Program and addresses many Critical Path Roadmap Questions related to: 1) bone loss, 2) bone fracture risk, 3) connective tissue, joint cartilage and disc maintenance, 4) muscle atrophy, posture and locomotor control, 5) multisystem (cross-risk) alterations, and 6) nutrition. This proposal provides a comprehensive, integrative, multisystem plan to transition a well-documented, ground-based countermeasure to space flight by the end of our three year grant. We will develop an optimal exercise protocol for musculoskeletal and other physiologic systems.

4. SPECIFIC CRITICAL ROADMAP QUESTIONS ADDRESSED BY OUR PROJECT

Our project has helped address the following critical questions:

2.07 We documented the benefits of an optimal LBNP treadmill exercise during exposure to simulated microgravity to minimize decreases in bone mass with regard to workout duration, intensity, and frequency. Impact loading appears to be an essential element.

2.09 An important predictor for bone loss during prolonged exposure to simulated microgravity (n-telopeptide) was reduced by LBNP treadmill exercise.

2.17 Spine connective tissue structure and function was maintained and pain reduced by LBNP treadmill exercise after prolonged simulated microgravity.

2.18 LBNP treadmill exercise can help reduce the incidence of muscle connective tissue injury and pain during recovery after prolonged microgravity.

2.26 LBNP treadmill exercise can help restore bone mass and geometry to their pre-bed rest state.

8.01 We have documented appropriate exercise prescription modalities and compliance factors needed to minimize losses in muscle mass, strength and endurance.

9.21 LBNP treadmill exercise can help counteract impairment of balance/equilibrium.

9.22 Our project has helped identify the relative contribution of orthostatic intolerance to post-flight neuromuscular coordination, ataxia, and locomotion difficulties.

9.24 LBNP treadmill exercise can help improve post-landing postural and locomotor control.

11.38 Our project has helped identify pre-landing and pre-egress performance and health parameters to assure adequate cardiovascular tone, neurological function, skeletal integrity, muscular strength, and endurance. Such tests include orthostatic tolerance by tilt/graded LBNP, sprint speed, balance/coordination, peak VO2, bone markers, muscle strength and DEXA.

5. NEW CRITICAL ROADMAP QUESTION GENERATED

What combination of LBNP treadmill exercise and resistive exercise can maintain cardiovascular, musculoskeletal and neurologic function during prolonged microgravity?

6. BACKGROUND

a. Mechanisms of Physiological Deconditioning in Space and Importance of Musculoskeletal and Cardiovascular Interactions

Mechanisms which underlie the loss of physiologic structure and function include: 1) reduced mechanical stresses and deformation of tissue, 2) loss of hydrostatic (gravitational) gradients within blood vessels and other fluid columns of the body, and 3) reduced afferent information (eg., sensory and proprioceptive feedback) and its central integration (Grigoriev and Egorov, 1988). These basic mechanisms alone or in combination help explain microgravity adaptations such as loss of bone mass and strength (Morey-Holton et al., 1996; LeBlanc et al.,1998), neuromuscular adaptations and muscle atrophy (Edgerton and Roy, 1996; Thomason and Booth, 1990; Roy et al., 1991; LeBlanc et al., 2000), myocardial atrophy (Perhonen et al., 2001), and vestibular dysfunction (Daunton, 1996).

Recent evidence is mounting that interactions between the cardiovascular system and the musculoskeletal system are very important for maintenance of bone and skeletal muscle. For example, fluid flow within bone, driven by both blood pressure and mechanical loads, generates shear stresses which play a major role in signal transduction and load-induced remodeling of bone (McAllister et al., 2000). In fact, femoral vein ligation in the hindlimb-suspended rat model increases bone mass and suggests that bone fluid flow modulates bone remodeling independent of mechanical loads (Bergula et al., 1999). Pulsatile fluid flow reduces alkaline phosphatase mRNA expression within one hour and decreases alkaline phosphatase enzyme activity after 24 hours, demonstrating that the bone fluid flow alters gene expression in osteoblasts (Hillsley and Frangos, 1997). Furthermore, bone fluid shear stress is the most potent stimulus of nitric oxide release in osteoblasts so far reported (Johnson et al., 1996). Thus, nitric oxide released by a fluid flow mechanism may play a primary role in bone maintenance.

Alterations of blood pressure and flow also affect blood vessel structure and function and in turn, affect bone and skeletal muscle. For example, simulated microgravity increases cerebral artery myogenic tone (Geary et al., 1998) and reduces arterial contractility (Purdy et al., 1998). Important and pioneering studies by Delp and co-workers document that altered blood pressure and fluid shifts associated with hindlimb suspension of rats profoundly change local distributions of blood flow and bone mass (Colleran et al, 2000). For example, during 28 days of head-down tilt, a significant gain in the skull mass correlates with significantly elevated local blood flow, whereas significant losses of femur and tibial mass correlate with local reduction of blood flow. Furthermore, structural and functional remodeling of arterial microvasculature in hindlimb skeletal muscle (soleus and gastrocnemius) during simulated microgravity may be induced by reduced local blood pressure and wall shear stress (Delp et al., 2000).

All of the above results in bone and muscle support the concept that musculoskeletal tissues are highly dependent on changes in local blood pressure, blood flow and fluid shear stress associated with simulated microgravity. Therefore to be effective, an integrated countermeasure should provide mechanical loads, blood pressure (hydrostatic) gradients and afferent information similar to that provided during upright activity on Earth.

b. LBNP Provides Load Bearing Independent of Gravity

Historically, lower body negative pressure (LBNP) has been used as a cardiovascular stressor to sequester blood and other body fluids within tissues exposed to subambient pressure (Wolthius et al., 1974; Whedon et al., 1977). To relax lower extremity muscles and to prevent inward displacement of the human subject, a saddle is placed within the LBNP chamber. However, in this configuration, subjects frequently report saddle pressure and discomfort at relatively low levels of LBNP. With this knowledge in mind and with a hypothesis of using LBNP to create Earth-like musculoskeletal and vascular loads during exercise in microgravity, we predicted and then directly measured the footward forces generated during upright-standing and supine LBNP in the absence of a saddle (Hargens et al., 1991). Without the saddle, these forces are borne by the feet, legs and spine (using shoulder straps). Examples of the "weighting" effect of LBNP exercise are illustrated in our website: http://bones.ucsd.edu.

Presently, exercise protocols and equipment for astronauts in space are unresolved (Greenleaf et al., 1989b; Convertino, 1990), although recent calculations suggest that all exercise in space to date has lacked sufficient loads to maintain preflight bone mass (Whalen et al., 1988; Cavanagh et al., 1992; Davis et al., 1996; Morey-Holton et al., 1996). High impact, eccentric exercise has been recommended as a means of maintaining muscle and bone integrity during space flight (Dudley et al., 1991; Cavanagh et al. 1992). Bone remodeling studies of Whalen and co-workers (1988) and Goldstein and associates (1988) suggest that bone tissue maintenance is relatively insensitive to the frequency (or number of cycles) of loading per day. Although Russian cosmonauts run on a treadmill for 2-3 hours per day to prevent bone loss, their bungee-card loading apparatus is too uncomfortable to generate loads over 60-70% body weight (Whalen, 1993). Furthermore, blood pressure stimuli at their feet are abnormally low because gravitational blood pressures are absent with their treadmill hardware (Watenpaugh and Hargens, 1996). These factors help explain the 1-2% bone loss experienced by Cosmonauts on Mir during the first 6 months of exposure to microgravity (Smith et al., 1999). Recent studies of rats exposed to high-impact loads indicate that femoral biomechanical properties are greater than those for either low-intensity exercise rats or sedentary rats (Jarvinen et al., 1999). Also, gains in bone density produced by high-impact exercise were maintained by twice per week aerobic and step exercises in an 8-month randomized controlled study of women (Heinonen et al., 1999). High-impact exercise was also found to promote bone gain in female athletes (Taaffe et al., 1997). Because ground reaction forces during running are on the order of three times higher than walking, and because bone is more responsive to load magnitude than to load frequency (Whalen et al., 1988), we believe high-impact treadmill exercise such as that obtained with LBNP (Hargens et al., 1991); Boda et al., 2000) may be highly efficacious for prolonged exposure to microgravity.

We documented that static ground reaction force (GRF) in a LBNP chamber without saddle is a product of the body cross-sectional area at the waist seal (A_{xy}) and the pressure differential between the external ambient and internal chamber environments (ΔP), where ΔP = LBNP :

$$GRF = A_{xy}\Delta P + Ma_{z,cm}$$

where $A_{xy}\Delta P$ represents static force and $Ma_{z,cm}$ represents inertial forces associated with treadmill exercise. For the average male subject, an additional static GRF of about one equivalent body weight is generated for each 100 mm Hg of LBNP (Hargens et al., 1991). By doubling the waist seal area, we also demonstrated that this pressure can be halved to about 50 mm Hg to normalize both musculoskeletal and cardiovascular loads (Watenpaugh et al., 1994).

During upright-standing posture, ground reaction force increases linearly from each subject's body weight at approximately 2% initial weight per mm Hg LBNP. Likewise, in supine subjects, footward force increases linearly with LBNP. Theoretically, recumbency is more analogous to actual microgravity because there is an absence of body weight acting on the feet under initial conditions of ambient pressure within the LBNP chamber (the footward force vector is neither directed nor supplemented by Earth's gravity vector). However, the G_x force during supine LBNP exercise on Earth prevents a totally accurate reproduction of LBNP exercise during actual microgravity because of presence of Earth's Gx vector and associated need for leg and back supports in our bed-rest subjects. (Boda et al., 2000).

c. Gravitational Hemodynamics on Earth and during Microgravity

Adult humans spend about two-thirds of their existence in upright postures. During upright posture on Earth, blood pressures are greater in the feet than at heart or head levels due to gravity's effects on columns of blood in the body (hydrostatic or gravitational pressures, Hargens et al., 1992; Watenpaugh and Hargens, 1996). For example, mean arterial pressure at heart level is normally about 100 mm Hg, whereas that in the head is slightly lower (e.g. 70 mm Hg) and that in the feet is much greater (e.g. 200 mm Hg) (Katkov and Chestukhin, 1980). During exposure to microgravity, all gravitational blood pressure gradients (arterial, venous and microcirculatory) are lost so that blood immediately shifts to the chest and head tissues. Prior work indicates that the structure and function of blood vessels is maintained by transmural stresses associated with local blood pressures (Delp et al., 1993; Delp et al., 1995; Delp et al., 2000; Fung, 1991). Moreover, Edgerton and collaborators (1995) found that capillary density per fiber was 24% lower in muscle biopsies from astronauts exposed to only 5 and 11 days of microgravity. In addition, Buckey and co-workers (1996) found that astronauts who tended to faint while standing after space flight exhibited a relative inability to increase systemic vascular resistance during post-flight stand tests, suggesting that some form of vascular deconditioning in the legs may contribute to post-flight orthostatic intolerance. Presently, there is no exercise hardware available for space flight to provide additional (gravitational) blood pressure to tissues of the lower body. Recent findings in the hindlimb-suspended rat model indicate that maintenance of bone also depends on gravity-dependent distributions of blood pressure and flow (Colleran et al., 2000).

d. Integrated “Exercise Within LBNP” Countermeasure for Long-Duration Space Flight

Theoretically, an integrated countermeasure for extended exposure to microgravity should combine high loads on the musculoskeletal system (Whalen et al., 1988) with normal regional distributions of transmural pressure across blood vessels (Hargens, 1994). As previously stated, we documented that about 100 mm Hg LBNP with a conventional waist seal provides a footward force equivalent to 1g body weight (Hargens et al., 1991), and that this pressure is essentially halved to about 50 mm Hg by doubling the waist seal area (Watenpaugh et al., 1994). Also, we found that interstitial fluid pressure matches LBNP chamber pressure whereas local blood pressures are not affected by LBNP (Aratow et al., 1993b). A partial anti-gravity (“anti-LBNP”) suit with highest tissue compression below the waist and lowest compression at the thighs will prevent abnormal and excessive pooling of blood and interstitial fluids and also provide a more Earth-like gradient of vascular transmural pressure. Muscle contraction further increases intramuscular fluid pressure (Sejersted et al., 1984; Aratow et al., 1993a), so that the stress of LBNP is counteracted in activated muscle. In fact, dynamic leg exercise doubles LBNP tolerance, in part by skeletal muscle pumping of venous blood (Watenpaugh et al., 1993). Dynamic loads during supine exercise within a LBNP chamber provide inertial forces on the musculoskeletal and cardiovascular systems similar to those present during upright exercise on Earth (Table 1). By doubling the area of our waist seal, we maintained 1g ground reaction forces and IMPs while normalizing cardiovascular strain (heart rate) to levels equivalent to upright exercise (Hargens et al., 1994). At the foot, these accelerative loads may generate intermittent transmural pressure impulses of 200 mm Hg over and above the levels normally present with supine LBNP or quiet, upright standing posture on Earth. Consequently, dynamic exercise in LBNP provides inertial forces on musculoskeletal and cardiovascular tissues of the lower body equivalent to those for upright dynamic exercise on Earth (Hargens, 1994).

Table 1: Comparison of upright exercise-1G with supine exercise-LBNP at 100 mm Hg. Values represent means ± SE. (Murthy et al., 1994b)

Parameters	Upright Exercise-1G	Supine Exercise-LBNP
Ground reaction force (N)	701 ± 24	743 ± 37
Soleus IMP (mm Hg)	47 ± 7	55 ± 8
Heart rate (beats/min)	81 ± 3	99 ± 5*

*Significantly higher than Upright Exercise-1G, $p < 0.05$. Heart rate was equivalent to that for upright exercise in 1g when LBNP was reduced to about 50 mm Hg, maintaining 1g reaction forces and soleus IMP with the larger waist seal.

The concept of treadmill exercise during LBNP has developed over the past 15 years (Hargens et al., 1991; Schwandt et al., 1991; Hargens et al., 1992; Whalen and Hargens, 1992). Although treadmill exercise with bungee cords (about 2 h per day) was strongly advocated for cosmonauts during long-duration Mir missions, biomechanical loads on musculoskeletal tissues of the lower body are only about 60-70% of those present on Earth (Whalen, 1993). Furthermore, such exercise probably generates little of the Earth-like gravitational vascular pressures which maintain blood vessel structure and function in the lower body (Hargens, 1994). Somewhat higher loads have been used with Thornton treadmills on Skylab (Thornton and Rummel, 1977) and Shuttle (Thornton, 1990; Whitmore and Turpin, 1992), but astronauts report discomfort where the bungee cord harness compresses shoulder and pelvic regions. Cavanagh and co-workers (1992 and Davis et al., 1996) have developed a vertical treadmill and human-subject suspension system in ground-based studies to simulate exercise in microgravity. However, they also used a bungee-cord tether system which can only be tolerated for short periods of exercise at 1g loads. We have recently tested our vertical treadmill within a LBNP chamber and found that one can comfortably run on the treadmill for well over 40 min daily at up to 1.2 body weights (~60 mm Hg) with partial compression shorts over the lower abdomen as a prophylaxis to prevent excessive blood pooling or herniae in the lower abdomen. The results presented in our Progress Report demonstrate the feasibility of proceeding with the additional bed rest studies on female identical twins and development of a flight-certified, inflatable LBNP chamber for subsequent tests on the Shuttle as proposed in this application.

B. MATERIALS AND METHODS

Eight sets of female and male identical twins were selected following a thorough medical examination to ensure their suitability for a safe and well-controlled study. Acceptable subjects were thoroughly briefed by the investigator team and provided informed, written consent before participating in this study.

Subjects were randomly divided into two groups to investigate the mechanism of action and efficacy of our partial vacuum exerciser concept. These 30 day bed rest studies were chosen to approximate longer-term microgravity exposures. Our previous shorter-term bed rest studies achieved significant results with seven subjects. We also examined two more sets of male twins, giving us a total of 8 sets of male twins. By coin toss selection; one twin exercised while the other twin did not exercise. The exercising twin ran in the supine LBNP chamber for 40 minutes plus a 5 minutes static LBNP period. They exercised once a day at 1.0 to1.2 body weight of footward force (approximately 50 - 60 mm Hg LBNP), their sibling was a non-exercise "control" subject. The interval exercise protocol was similar to that employed by us in our previous two week studies: 7 min warm-up at 40% peak oxygen uptake, followed by 3 min at 60%, 2 min at 40%, 3 min at 70%, 2 min at 50%, 3 min at 80%, 2 min at 60%, 3 min at 80%, 2 min at 50%, 3 min at 70%, 2 min at 40%, 3 min at 60%, and 5 min cool-down at 40% peak oxygen uptake (40 min total) with an additional 5 min of supine, stationary exposure to 50 mm Hg LBNP. Exercise bouts occurred at the same time of day for each subject in the exercise group. Physiologic and other tests for both groups were similarly controlled in terms of timing during the day.

A 3 day orientation period occurred prior to the start of bedrest to acquaint subjects with our facilities, personnel, procedures and experiments, including orthostatic tolerance, muscle strength and exercise capacity tests. All subjects reported to the UCSD GCRC on their designated start day. Subjects were discharged from the GCRC about 39 days later.

In the baseline control period, activity logs were maintained on each subject, and ambulatory levels of plasma and urinary markers of bone loss were measured on the three days prior to bedrest. An aliquot was taken from all voids, or at least all 24 hour pools, to ensure that we can back fill if necessary. All physiologic tests took place at the same time of day for a given subject. These tests were staggered so that sufficient time is allowed to complete each procedure. While the subjects live at the GCRC, their daily sodium intake was controlled at approximately 170 mEq (about 3.5 g per day) and their diet also was controlled (approximately 2500-3000 kcal per day, depending on exercise level). Their body weight, fluid intake, and urine output were monitored on a daily basis. Our subjects maintained a neutral or positive fluid balance, and they did not lose more than 1kg weight when performing as a control or LBNP exerciser during 30 day HDT. During the entire period of bedrest, all subjects remained in 6° HDT except during periods for bath/shower and exercise (0.5-1.5 h/day), when they were horizontal (0°).

In the baseline control period, the Hamilton Depression Rating Scale and Beck Depression Inventory were administered to each subject to assess pre-study depressive symptomatology. These tests were re-administered during the last week of the bedrest period.

Prior to initiation of HDT, plasma volume, leg and spinal muscle strength, knee laxity, DEXA, calcaneal ultrasound, MRI volumes, heart muscle mass, tibial stiffness, arm venous pressure, plasma and urinary bone markers, creatinine, and collagen cross links were measured in all subjects (Table 2). On the day before initiation of HDT, all subjects were tested for orthostatic tolerance, postural stability, gait parameters, and upright peak oxygen uptake. During the recovery period, these tests were repeated within 4 h of return to upright posture. Subjects started and end the study at a rate of two per day. Subjects were staggered with respect to time of HDT initiation and return to upright posture to facilitate testing. Subjects were active and take controlled walks during control days and recovery days. Experimental stations included: 1) leg strength, 2) tibial stiffness, 3) orthostatic tolerance (includes plasma volume and arm venous pressure), and 4) posture/gait analysis and peak upright exercise capacity. There was also be a station specifically for LBNP exercise.

Table 2. Daily order of events

Day required	Procedure (method from pp 7-18)	Time
Familiarization day 1	Report to GCRC, check in	15 min
	Orthostatic tolerance (c,d,e,f,g,h,i,k,t,u)	90 min
	Peak upright exercise capacity (l)	1 hr
	Noninvasive ICP (t)	30 min

Familiarization day 2	Muscle strength (m)	90 min
	LBNP exercise for selected twin	90 min
Familiarization day 3	Plasma volume and hematocrit (j)	30 min
	Regional arterial compliance (u)	15 min
	Echocardiography (y)	1 hr
Control day 1	Serum and 24 hr urine collection (a)	30 min
	Discomfort questionnaire (r)	15 min
	Orthostatic tolerance (c,d,e,f,g,h,i,k,t,u)	90 min
	Sprint speed (o)	10 min
	Posture analysis (o)	20 min
	Peak upright exercise capacity (l)	1 hr
Control day 2	Serum and 24 hr urine collection (a)	15 min
	Discomfort questionnaire (r)	15 min
	DXA (b)	1 hr
	Muscle strength (m)	90 min
	Regional arterial compliance (u)	15 min
	Sleep quality	9 hr
Control day 3	Serum and 24 hr urine collection (a)	30 min
	Discomfort questionnaire (r)	15 min
	Spinal compression and heart mass (h)	1 hr
	Heel ultrasound (b)	10 min
	Hamilton and Beck tests (w)	30 min
	Knee Laxity (s)	30 min
	Sleep quality	9 hr
Bedrest day 1	Begin bedrest	
	Discomfort questionnaire (r)	15 min
Bedrest (all days)	Discomfort questionnaire (r)	15 min
Bedrest	Exercise (all days except 7,14,21,28,30)	90 min
Bedrest 4,11,18,25	Noninvasive ICP (t)	30 min
	TCD (d)	30 min
Bedrest 5,12,19,26	Serum and 24 hr urine collection (a)	30 min

Bedrest 17/18,26/27 sleep	Sleep quality (v)	9 hr during
Bedrest 14	Regional arterial compliance (u)	15 min
Bedrest 24	Hamilton and Beck test (w)	30 min
Bedrest 25	N/A	
Bedrest 26	Knee laxity (s)	30 min
Bedrest 27	N/A	
Bedrest 28	Spinal compression and heart mass (h)	1 hr
Bedrest 30	Plasma volume and hematocrit (j)	30 min
	Echocardiography (y)	1 hr
	Regional arterial compliance (u)	15 min
Recovery day 1	Serum and 24 hr urine collection (a)	30 min
	Discomfort questionnaire (r)	15 min
	Orthostatic tolerance (c,d,e,f,g,h,i,k,t,u)	90 min
	Sprint speed (o)	10 min
	Posture analysis (o)	20 min
	Peak upright exercise capacity (l)	1 hr
Recovery day 2	Serum and 24 hr urine collection (a)	15 min
	Discomfort questionnaire (r)	15 min
	DXA (b)	1 hr
	Muscle strength (m)	90 min
Recovery day 3	Discomfort questionnaire (r)	15 min
	Heel ultrasound (b)	10 min
	Knee laxity (s)	30 min
	Ambulation, discharge from GCRC	1 hr

a. Analyses of Bone Loss by Serum and Urine Markers

Biological Sample Collections

Fasting (>10 hr) blood samples for endocrine and other biochemical measurements will be collected immediately after crewmembers awaken, at the same time of day, to minimize the effect of diurnal changes in endocrine and biochemical markers (Gundberg et al., 1998). Each of the biochemical/endocrine profile determinations requires 15 ml of blood.

Urine samples will be collected as separate voids into individual bottles. The volume of each will be determined, and a 24-hour pool will be prepared using standard techniques. Aliquots will be removed for each void for indices requiring void-by-void processing (calcium, calcium isotopes, and creatinine). Total volume and pH will be determined on the 24-h pool, and aliquots will be removed for biochemical and endocrine analytes. These will be processed and frozen until analysis. Although urine specimens will be collected throughout the study, assays will be carried out only on selected specimens per **Table 2** to reduce cost. Wherever possible, samples will be stored at -70°C and assayed at the same time to minimize inter-assay variations in the results.

<u>Endocrine and Biochemistry Measurements</u>

Endocrine/biochemical profile includes serum and urine indices of bone and calcium metabolism. Urine indices will be measured on samples from 2 consecutive days during each of the sessions to reduce cost. Urine will be collected throughout the study, but complete analyses will be run on indicated samples to reduce costs.

Circulating bone- and calcium-related factors [e.g., parathyroid hormone (PTH), vitamin D metabolites] will be assessed in serum. PTH will be assayed for the intact peptide by radioimmunoassay (RIA). Intact PTH, while only about 10% of the circulating PTH fragments, is the assay of choice because the clearance of intact PTH is largely independent of renal function (Breslau, 1992). Serum 1,25-dihydroxyvitamin D will be measured by radioimmunoassay after extraction of samples with acetonitrile and purification on $C_{18}OH$ cartridges (Hollis, 1986; Lambert et al., 1978). Serum 25-hydroxyvitamin D will be determined by radioimmunoassay after extraction with acetonitrile (Haddad and Chyu, 1971).

Markers of bone formation, including bone-specific serum alkaline phosphatase (BSAP) activity and osteocalcin, will be measured. Levels of undercarboxylated osteocalcin will also be determined, as this is a marker of the vitamin K status of the individual, which can have implications for bone health. Serum total (Gundberg et al., 1984) osteocalcin will be determined by radioimmunoassay. BSAP will be determined by enzyme-linked immunoassay (Farley et al., 1993; Rosalki et al., 1993).

Urine samples will be analyzed for collagen crosslinks using commercially available kits (PyrilinksJ and PyrilinksJ-D, Quidel, Inc. Santa Clara, CA; Osteomark7 ELISA kit, Ostex International, Inc., Seattle, WA), as previously reported (Smith et al., 1998; Smith et al., 1999). These widely used markers of bone resorption are extremely reliable when evaluating within-subject interventions (e.g., spaceflight, countermeasure use) (Smith et al., 2003).

Urine and serum calcium will be determined using inductively-coupled plasma mass spectrometry (ICP-MS) (Hsiung et al., 1997). Urine volumes will be monitored and recorded daily for each subject.

b. DEXA and Heel Ultrasound

Bone density and body composition will be measured using dual-energy X-ray absorptiometry (DEXA) (Sabatier and Guaydier-Souquieres, 1989). Total body calcium [including bone mineral content (BMC), projected area (cm^2), and bone mineral density (BMD), lean body mass (kg), and body fat content (kg, including percentage] will be determined from total body DEXA scans on each subject before and after 180 days of flight. High-resolution regional scans of the lumbar spine (frontal projection) and proximal femur (non-dominant side) will be conducted, including measurement of BMC, projected area, and BMD for each region of interest. If available, a Lunar Corporation DPX-IQ DEXA system will be employed for these densitometric studies. Calcaneal ultrasound tests will be performed using standard clinical technique with a Hologic Sahara device or equivalent (Laugier et al., 1996).

c. Leg and Spine Muscle Strengths

Maximal isokinetic torque will be determined using MARES equipment that will be available in 2005. Dynamometer positions and range of motion will be standardized during the familiarization sessions for each subject and held constant for all repeat tests. Maximal isokinetic torque testing has been performed previously in our bed rest studies using a protocol developed at NASA JSC (Bamman et al., 1997; Bamman et al., 1998).

d. Sprint Speed, Posture, and Gait Analysis

We hypothesize that LBNP treadmill exercise during bedrest will maintain sprint speed at pre-bedrest levels. Subjects will sprint a distance of 50 m from a standing start and will be timed with a stopwatch. Two sprint trials will be performed before bedrest, and two trials will be performed at approximately 10 min after arising from bedrest. We will select the faster of the two trials as sprint speed at each time.

After landing the Shuttle, crewmembers often have trouble ambulating due to impaired postural equilibrium and gait. These microgravity-induced deficits may be caused by reduced otolith organ and lower-extremity proprioceptive inputs, weakened leg muscles, altered neuromuscular control, and/or orthostatic hypotension. In our recent bedrest studies, our LBNP exercise evaluation did maintain posture and gait parameters at pre-HDT levels (Boda *et al.*, 1997, 2003). The tests that we propose will also provide quantitative data on possible mechanisms of this maintenance of performance.
Balance control performance will be evaluated on multiple occasions before and after flight using a computerized dynamic posturography system (CDP; Equitest, NeuroCom International, Clackamas, OR; Paloski et al, 1999). The CDP protocol will test balance control during 20-second epochs of quiet stance with normal and altered sensory reference conditions. Subjects will be tested approximately 60, 30, and 10 days before flight and five times after landing (R+ 0, 1, 3, 5, and 10 days after short duration missions and R+ 1, 3, 5, 10, and 20 days after long duration missions). The standardized CDP test battery comprises six sensory organization tests that combine three visual conditions (eyes open, eyes closed, and sway-referenced vision) with two proprioceptive conditions (fixed and sway-referenced support surfaces; Nashner, 1993). The standard protocol will add enhanced trials requiring subjects to perform condition 5 (absent vision and dynamically altered somatosensory reference information) with static and dynamic head tilts. During static head tilt trials, subjects will attempt to maintain head erect (static control condition) or tilted by ±30° (extension, flexion). During dynamic head tilt trials, subjects will attempt to perform continuous ±30° sinusoidal head oscillations (paced by an audible tone) at a frequency of 0.33 Hz (Paloski et al., 2004).

e. Exercise Capacity and Peak Oxygen Uptake

We measure $V0_2$ peak to assess aerobic capacity and to determine exercise prescription. A graded exercise test will be designed individually for each subject such that three 3-min submaximal stages will be completed during level running (Lee, 1997; Watenpaugh et al., 2000). Oxygen uptake (VO_2), heart rate (HR), expired ventilation (V_E), and respiratory exchange ratio (RER) will be determined for each submaximal stage in steady state. Peak oxygen consumption ($VO_{2\ peak}$) will be determined as the highest level of VO_2 measured during a 1-min period in which at least two of the following criteria are met: RER exceeds 1.1, maximal HR is greater than 85% of the age-predicted HR maximum, and/or a plateau of the VO_2 curve occurs.

f. Leg, Spine and Heart Muscle Volumes and Spinal Compression Test

While the subjects are in the MRI, we will also scan muscles of the leg (soleus and gastrocnemius), spine (iliopsoas) and heart to determine their muscle volumes using procedures previously developed for ground and flight studies (LeBlanc et al., 2000; Kimura et al., 2001; Perhonen et al., 2001). While noninvasive imaging systems such as magnetic resonance imaging (MRI) and computed tomography scanners have been used as diagnostic tools for studying back pain and orthopaedic injury, these imaging devices require patients to lie down, thus eliminating axial loads associated with upright posture (see Fig. 13 below). The MRI-Compatible Spinal Compression Harness (DynaMed, Inc.) is designed to produce gravity-like axial loads on the body. Our tests will measure curvature and disc height changes with 50% body weight loads on the spine (Hargens et al., 1998).

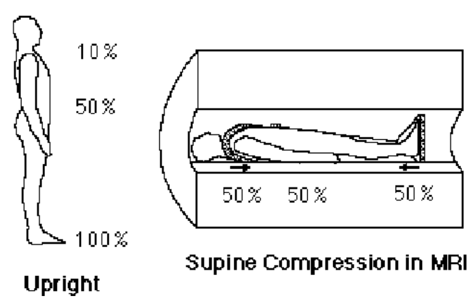


Figure 1. Percentage body weight locally supported during upright posture (left) and supine compression in MRI (right). In upright posture, a gradient of weight bearing exists from the head to the feet, whereas the harness compresses tissues uniformly between the sites of compression. Thus, loads are adjusted to the specific tissue to be scanned under load (e.g. lumbar spine typically experiences 50% body weight in upright posture).

In our spinal compression tests, we will load the lumbar spine to 50% body weight and measure total spinal length, spinal curvature, lumbar disc height, and disc water content before and after a 20 minute compression period (Kimura et al., 2001).

g. Orthostatic Tolerance Test

As performed in our lab previously (Watenpaugh, 2001; O'Leary, 2001), we will conduct orthostatic tolerance tests to presyncope before and after space flight. We employ a combination of 60° head-up tilt and graded increases of LBNP. This combination imposes stepwise challenge of orthostatic cardiovascular control and provides a time-efficient means of reliably bringing all subjects to a presyncopal test endpoint. We expect that crewmembers will maintain their orthostatic tolerance better with LBNP exercise than they would without the countermeasure. Heart rate (ECG), arm (auscultation) and finger (Finapres) arterial blood pressure, and middle cerebral artery blood flow velocity (transcranial Doppler ultrasound) will be monitored during orthostatic tolerance tests.

h. Plasma Volume and Hematocrit

Because plasma volume contraction and reduced RBC mass are known manifestations of microgravity-induced deconditioning, plasma volume and hematocrit are essential parameters for evaluating the efficacy of a countermeasure. Plasma volume will be determined by carbon monoxide rebreathing (Burge and Skinner, 1995; Thomsen et al., 1991). Hematocrit will be measured with a Micro-capillary Reader (Damon/IEC Division) after 4 min of microhematocrit centrifugation.

i. Statistical Analyses

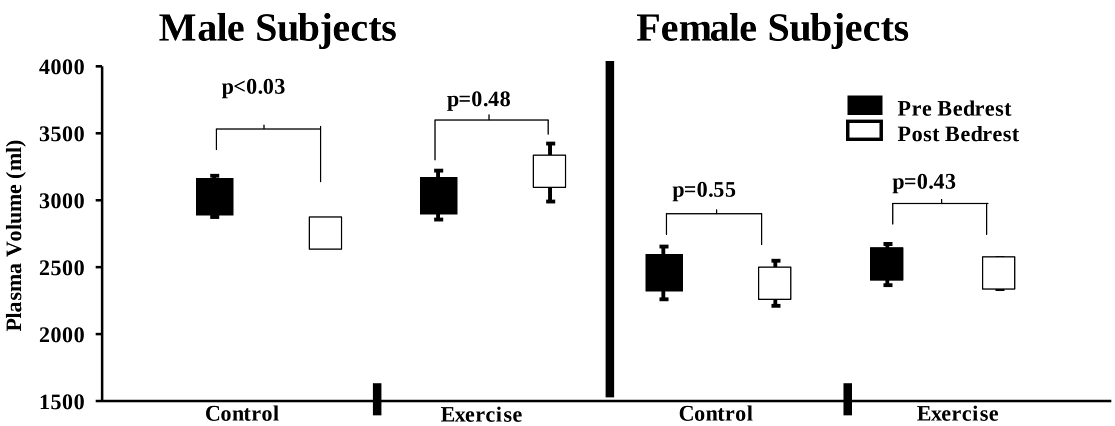
Based upon our previous 5, 15 and 30 day bed rest studies of twin and non-twin subjects, we have reached statistical significance ($p < 0.05$) for most tests by comparisons of 8 exercise versus 8 control subjects. Statistical analysis will include three factor repeated measures ANOVA to assess flight, countermeasure, and interaction effects. When ANOVA results indicate statistical significance for a given parameter, post hoc analyses (Fisher LSD or Bonferroni t-tests) will isolate specific significant findings. When a non-normal distribution is suspected (such as with visual-analog data), non-parametric analyses will be performed using Mann-Whitney and Wilcoxon Rank Sums tests. Regression analyses of relevant variable pairs will determine the extent of correlation. Significance level will be set at $p < 0.05$.

C. RESULTS

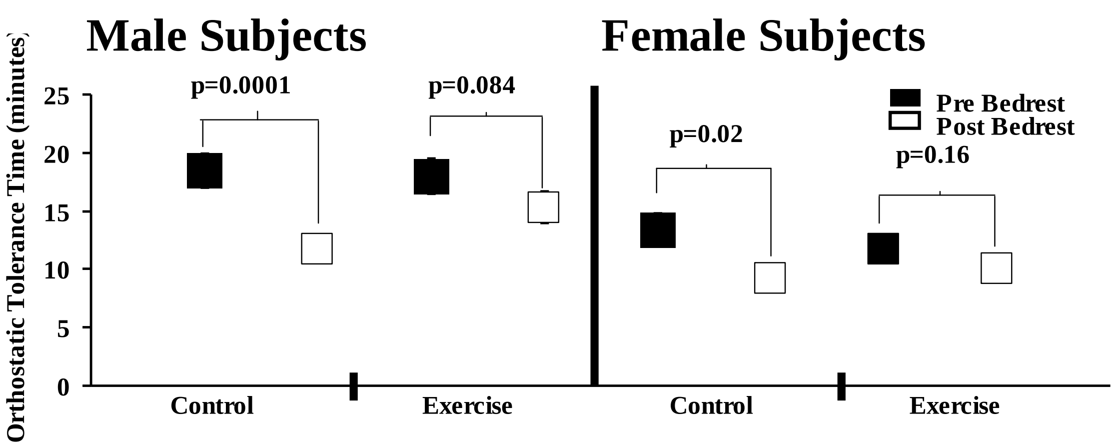
Orthostatic tolerance (time to pre-syncope during tilt and graded LBNP), plasma volume, and sprint speed decreased significantly ($p<0.05$) after 30 days bed rest in the CON group, but was relatively maintained in the EX group. Upright VO_2pk, muscle strength of knee and spine, and endurance decreased significantly in CON group, but were preserved in the EX group. Also, the EX group had normal spinal compressibility with 50% BW axial load and significantly higher back muscle strength after bed rest than the CON group. Urinary n-telopeptide (NTX) excretion, an index of bone resorption, was increased during bed rest in CON, but not in EX subjects. However, markers of osteoblast activity were similar in the EX and CON groups.

Our treadmill exercise protocol within LBNP maintains plasma volume, orthostatic responses, upright exercise capacity, muscle strength and endurance during bed rest. These results document the efficacy of our exercise countermeasure in both males and females. LBNP exercise counteracts increased osteoclast activity, but resistive exercise may be needed to increase bone formation. Future studies will include a combination of supine treadmill exercise in LBNP and flywheel resistive exercise during the International Long-Term Bed Rest Project. Treadmill exercise in LBNP may be an early, low mass, low power and efficacious form of artificial gravity for exploration missions. However, our LBNP exercise hardware must be redesigned for space flight and crew habitability.

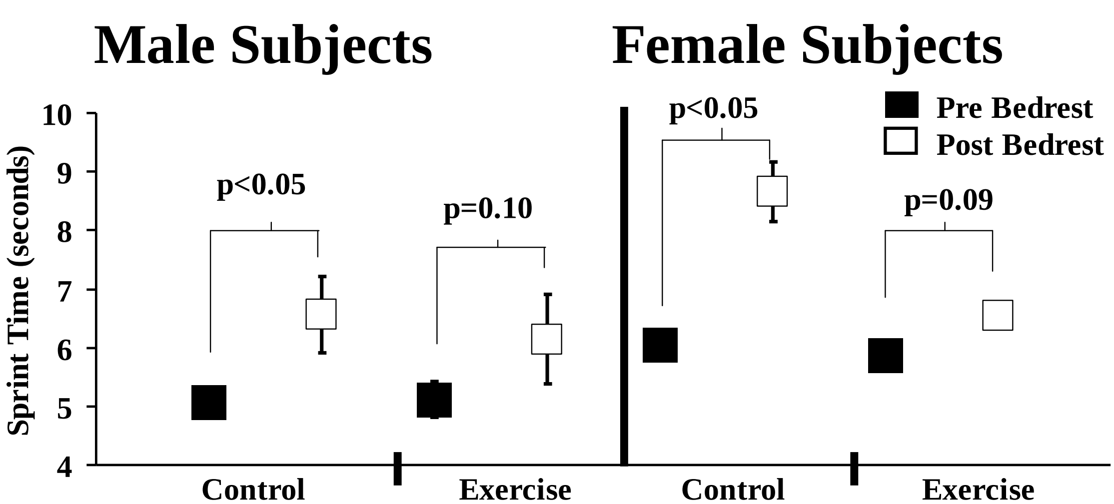
Plasma Volume (Mean ± SE)



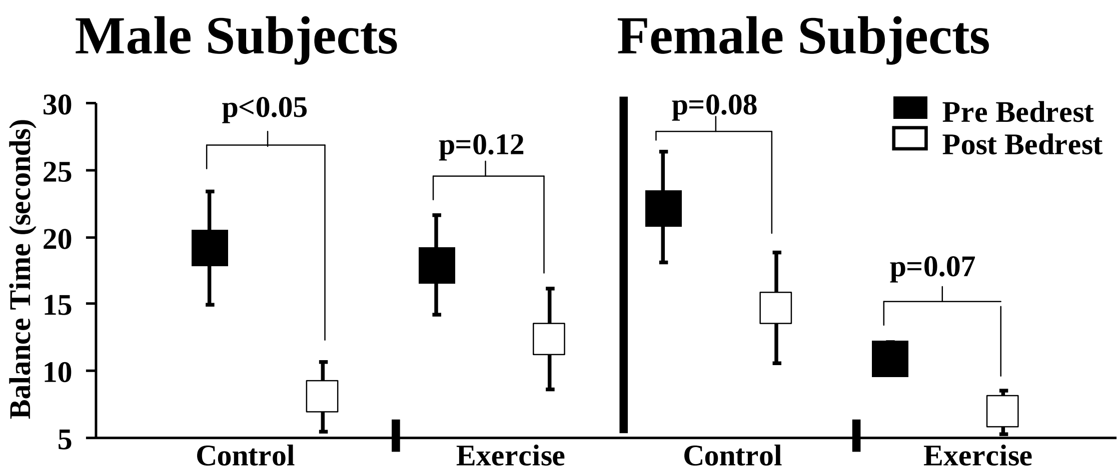
Orthostatic Tolerance
(Mean ± SE)



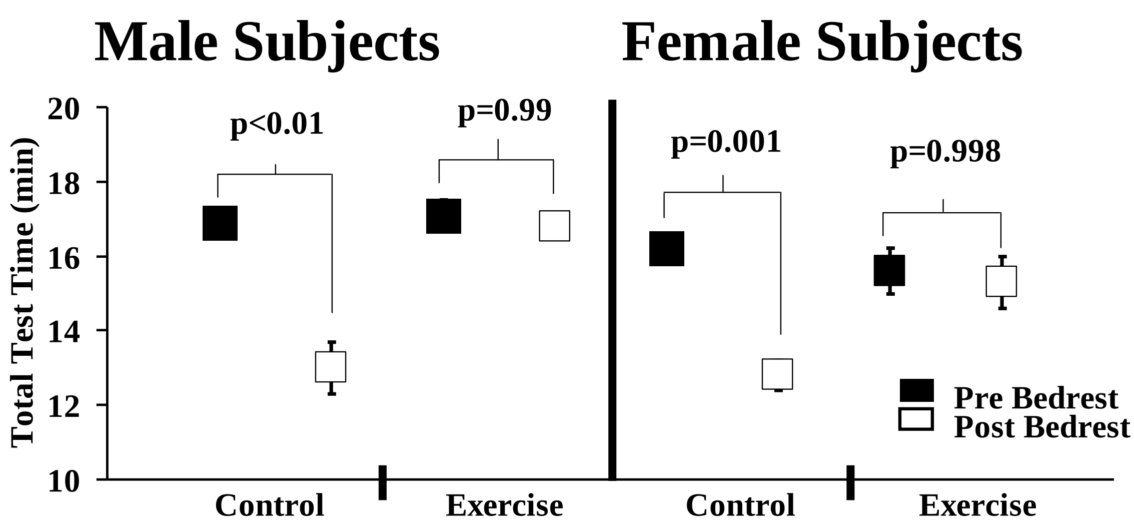
Sprint Time (Mean ± SE)



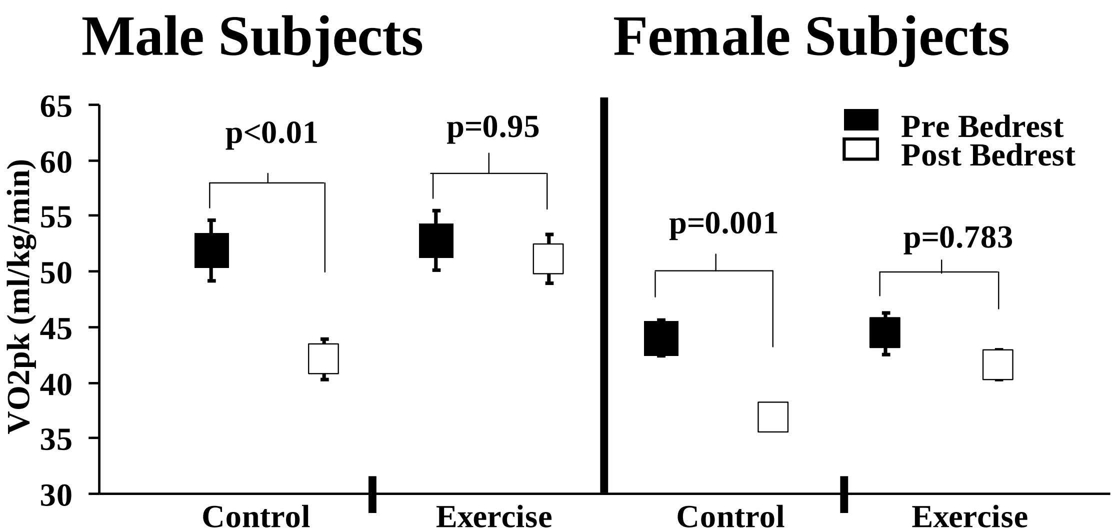
Balance: Right Eye Closed
(Mean ± SE)



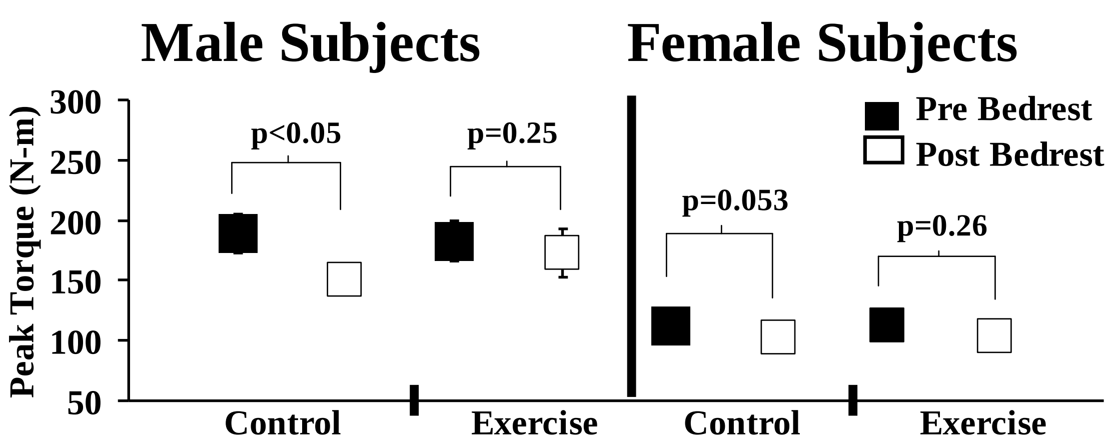
Exercise Capacity: Total Test Time
(Mean ± SE)



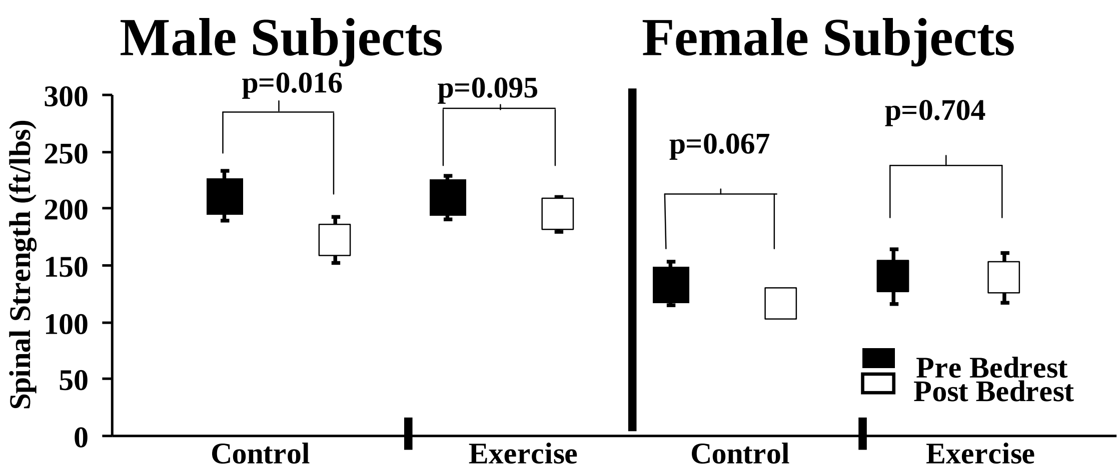
Upright Peak Oxygen Consumption
(Mean±SE)



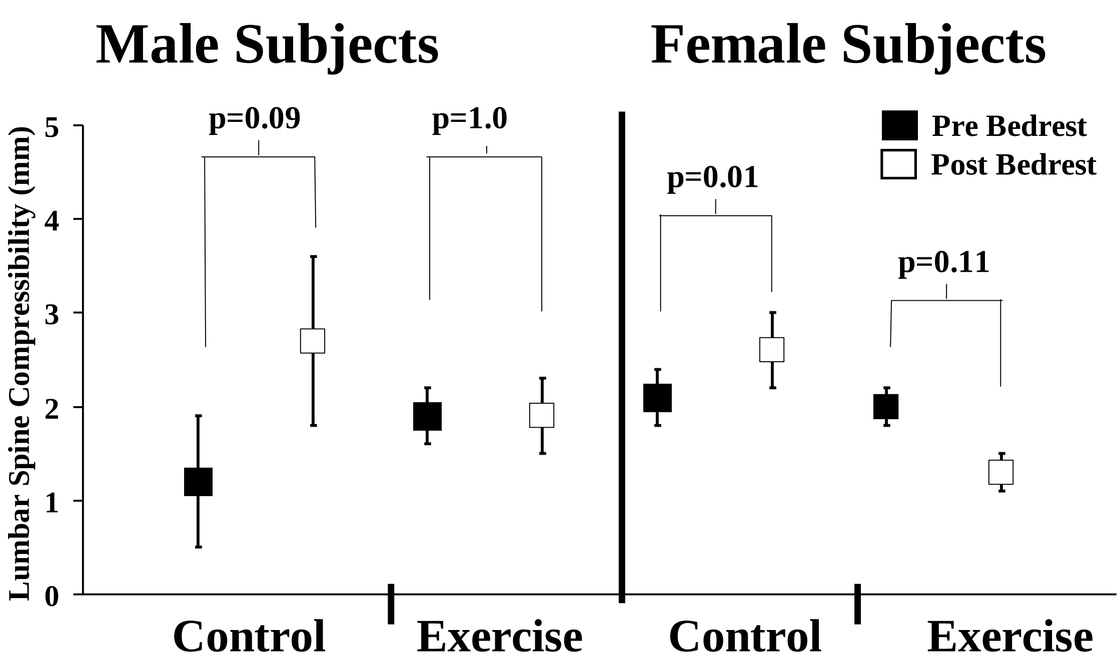
Isokinetic Knee Strength
(Mean±SE)

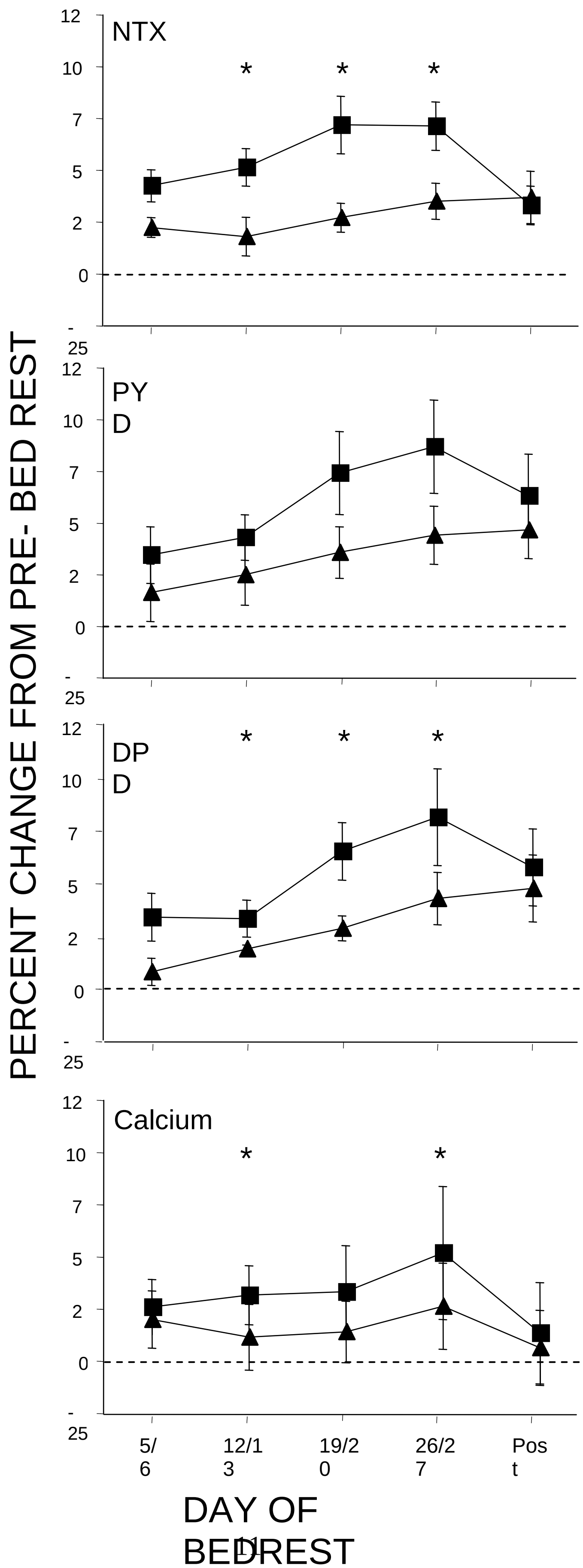


Spine Strength
(Mean ± SE)



Lumbar Spine Compressibility with 50% BW load (Mean ± SE)





PERCENT CHANGE FROM PRE- BED REST
NTX
PY
D
DP
D
Calcium
12
10
7
5
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0
-
25
*
5/
6
12/1
3
19/2
0
26/2
7
Pos
t
DAY OF
BEDREST

Summary

Our LBNP protocol (40 min treadmill exercise + 5 min static LBNP for 6 days/week) maintains:

- Plasma volume
- Orthostatic tolerance
- Sprint speed and balance
- Upright exercise capacity
- Leg strength
- Spinal function and spinal muscle strength
- Some bone and calcium metabolism parameters
  But some male responses differ from females

Conclusions

- Countermeasure provides comfortable,
  high-intensity exercise to preserve many physiologic systems over 30 days of simulated microgravity
- LBNP exercise may be an early, low power, low mass, and cost effective alternative to centrifugation for Exploration Missions
- Results for women are somewhat inconclusive
- User friendly and fun?

Improvements Proposed

- Longer period of static LBNP to improve orthostatic tolerance
- Resistive exercise to increase osteoblastic activity and muscle strength
- More studies of women at MEDES in 2005
- VE to bring “home/g perception” to space exercise

NASA "Spin-Offs" for Earth Benefit

"Artificial gravity" concept was applied to:

- Enhance upright athletic performance on Earth
- Improve rehab of orthopaedic and other patients by providing carefully graded buoyancy using LBPP
- Promote healing and return to ambulation earlier than current rehab modalities
- Understand heritability of physiologic traits such as orthostatic tolerance, oxygen consumption, muscle strength, bone density, renal stone risk

D. DISCUSSION AND SIGNIFICANCE

Our finding of the magnitude and mechanism of force production by LBNP has important implications for simulating gravity in space and increasing weightbearing on Earth without the use of a centrifuge. The upright-standing results indicate that ground reaction force increases by approximately one equivalent body weight for each 50 mm Hg LBNP. The supine data suggest that during microgravity, uniaxial loading of lower-body tissues can be comfortably produced by a similar level of LBNP. Furthermore, the lower-body musculoskeletal loss experienced by crew exposed to long-term space flight (Thornton and Rummel, 1977; Whedon et al., 1977; Schneider et al., 1989; LeBlanc et al., 1998; LeBlanc et al., 2000) may be prevented by safe levels of high-intensity, short-duration exercise within such a chamber (Hargens et al., 1991; Boda et al., 2000; Watenpaugh et al., 2000). The use of a different air pressure separating the upper and lower body, such as proposed in this project, distributes the net force uniformly over the entire upper surface of the body. This concept thereby avoids the discomfort of localized high pressures typical of bungee cord harness systems.

Variations of blood pressures due to inertial loads with normal gait have been documented in humans (Noddeland et al., 1983; Pollack and Wood, 1949) and other animals (Hargens et al., 1987b), and such variations are important for maintenance of normal vascular structure and function in dependent tissues (Delp et al., 1993; Delp et al., 1995; Fung, 1991). Recent evidence points to the importance of blood pressure gradients for maintenance of bone and skeletal muscle as well (Colleran et al., 2000; Delp et al., 2000). LBNP exercise simulates gravitational blood pressures in the lower body circulation, and permits the simultaneous additional impact loading of lower body tissues and blood vessels during exercise. No other exercise equipment previously recommended for crew members, other than exercise on a long radius centrifuge, provides this level of simultaneous musculoskeletal and transmural vascular stress. On Earth this concept of loading could also be in the lower body applied to individual limbs for rehabilitation purposes, such as enhancing bone formation after fracture.

Muscle atrophy, loss of bone and collagen mass/strength reduced exercise capacity and orthostatic intolerance during microgravity may be due to reduced loading history and loss of circulatory gravitational pressure gradients compared to those on Earth. Therefore, exercise within a LBNP chamber which provides inertial fluid pressures during space flight may reproduce the functional interdependence of the musculoskeletal and cardiovascular systems during normal daily activities on Earth (Fig. 2). A treadmill or other exercise equipment can be placed within the chamber to provide inertial forces, high resistance, and eccentric-type training exercises (Dudley et al., 1991; Hargens et al., 1989b) that may help to maintain circulatory tone, bone mass, and muscle mass. Although not a force like gravity, the resultant force from the air pressure resembles the action of gravity in several important ways. Most importantly, the center of pressure (the location of the resultant force) is at or very near the center of mass of the body (and center of gravity on Earth) during ‘upright’ activities. Also, the applied force throughout the gait cycle is relatively constant, because the displaced volume during exercise is small compared to the total chamber volume. If the chamber seal is air-tight and adiabatic, the device acts as a constant-force, energy-conserving spring. Because the air pressure is uniformly distributed over the surface of the body, the force is not detected as a localized force pulling or pushing down on the body.

In addition to the potential musculoskeletal and cardiovascular benefits, treadmill exercise within LBNP probably activates the same neuromuscular systems as normal ambulation, which should help prevent loss of neuromuscular coordination following bed rest (Haines, 1974; Cohen, 1989; Dupui et al. 1992) and space flight (Homick et al., 1977; Young et al., 1984; Reschke et al., 1984; Grigoriev and Egorov, 1988; Kozlovskaya et al., 1990). During bed rest studies, neuromuscular deconditioning degrades gait parameters, reducing step length, walking speed, and balance stability, all of which last up to three days after bed rest (Haines, 1974; Cohen et al., 1986; Dupui et al. 1992).

It is possible that short periods of intense effort may optimize the benefit/time ratio for maintaining health and performance of crew members in space. Exercise within a LBNP chamber in space may provide a safe, inexpensive, energy efficient, and compact alternative to centrifugation (without Coriolis effects) for many physiologic systems during long-duration existence in microgravity.

E. OTHER INFORMATION

1. STUDENT AND POSTDOC AWARDS

One of our undergraduate students, Eli Groppo, won the First Place Award at the Southwest ACSM Student Competition, even though he competed against masters and doctoral level students (see Groppo E, R Eastlack, A Cutuk, H Noh, A Langemack, E Quigley, G Steinbach, A Hargens and R Pedowitz. Rehabilitation Using Lower Body Positive Pressure After Knee Surgery To Decrease Load And Preserve Gait Mechanics. Presented at the Southwestern American College of Sports Medicine Conference, **1st Place Student Award**, San Diego, California, 2000). In 2002, another undergraduate student, Brandon Macias, won a Student Award at the Southwest ACSM meeting for his exercise training research (see Macias B, H Tran Cao, B Lee, M Bawa, E Groppo, R Pedowitz, A Hargens. Physiologic responses of lower body negative pressure treadmill exercise as a training modality. American College of Sports Medicine (ACSM) SW Chapter, Las Vegas, Nevada, 2002, **Student Award from American College of Sports Medicine, SW Chapter, Las Vegas, Nevada, 2002)**.). Also, in 2002, Eli Groppo received a Zweifach Student Award from the Microcirculatory Society for a new neutrophil activation assay (see Groppo E, R Ng, R Eastlack, A Cutuk, G Schmid-Schonbein, A Hargens and R Pedowitz. Quantifying neutrophil activation with xylenol orange reaction: a possible method for detecting systemic activation. FASEB Journal 16:A82(121.3), 2002 (**Zweifach Student Award from the Microcirculatory Society**). Another lab resident, Maneesh Bawa, received a First Place Award at the American Academy of Orthopaedic Surgeon's meeting in February 2003 (see Bawa, M, ER Groppo, GC Steinbach, SM Lee, SM Smith, AR Hargens, RS Meyer. Lower body negative pressure treadmill exercise prevents the spinal deconditioning of astronauts in simulated space flight. American Academy of Orthopaedic Surgeons Annual Meeting, New Orleans, Louisiana, 2003 **(1st Prize Award for the Rehabilitation and Spine Division at the American Academy of Orthopaedic Surgeons)**. Also, one of our students won a **First Place Student Award** (Keehan MM, SMC Lee, DE Watenpaugh, SM Schneider and AR Hargens. Simultaneous exercise and lower body negative pressure countermeasure during bed rest protects exercise capacity. 7th Congress of the International Society for Adaptive Medicine in 2003). and a postdoc won **Second Place for New Investigator Award (**Kimura S, GC Steinbach, DE Watenpaugh and AR Hargens. Lower body negative pressure treadmill exercise may maintain lumbar spinal structure and function during 30 days of bedrest. 7th Congress of the International Society for Adaptive Medicine, 2003). Finally, Bob Eastlack, a former lab resident, won the Jacquelin Perry Award for his lower body positive pressure research on knee surgery patients at the 2003 Orthopaedic Rehabilitation Association meeting. The paper was recently published (Eastlack RK, Hargens AR, Groppo ER, Steinbach GC, White KK, Pedowitz RA. Lower body positive-pressure exercise after knee surgery. Clin Orthop Relat Res 431:213-219, 2005).

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Award for the Rehabilitation and Spine Division at the American Academy of Orthopaedic Surgeons).

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Keehan MM, SMC Lee, DE Watenpaugh, SM Schneider and AR Hargens. Simultaneous exercise and lower body negative pressure countermeasure during bed rest protects exercise capacity. 7th Congress of the International Society for Adaptive Medicine, page 22, San Diego, California, 2003 **(First Place Student Award)**.

Kimura S, GC Steinbach, DE Watenpaugh and AR Hargens. Lower body negative pressure treadmill exercise may maintain lumbar spinal structure and function during 30 days of bedrest. 7th Congress of the International Society for Adaptive Medicine, page 23, San Diego, California, 2003. **(Second Place New Investigator Award)**

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